Phytochemistry, Vol. 26, No. 2, pp 467–470, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

FIVE GRINDELANE DITERPENOIDS FROM *GRINDELIA ACUTIFOLIA*

BARBARA N. TIMMERMANN, JOSEPH J. HOFFMANN, SHIVANAND D. JOLAD*, ROBERT B. BATES† and TERUNA J. SIAHAAN†

University of Arizona, Office of Arid Lands Studies, Bioresources Research Facility, 250 E. Valencia Road, Tucson, AZ 85706, U.S.A.; *Department of Pharmaceutical Sciences and †Department of Chemistry, Tucson, AZ 85721, U.S.A.

(*Revised received* 24 *April* 1986)

Key Word Index—*Grindelia acutifolia*; Asteraceae; Astereae; Solidagininae; diterpenoid acids; labdanes; grindelanes; naval stores.

Abstract—Five new labdane diterpenoid acids, 6α-hydroxy-17-acetoxygrindelic acid, 6-oxo-17-acetoxygrindelic acid, 6α-hydroxy-17-isovaleroxygrindelic acid, 6α-hydroxy-17-(α-methylbutyroxy)grindelic acid and 6-oxo-17-isobutyroxygrindelic acid, were shown to be present in *Grindelia acutifolia*, mainly on the basis of comparisons of the spectra of their methyl ester derivatives with those of their 6-deoxy derivatives, several of which are major constituents of this plant species.

INTRODUCTION

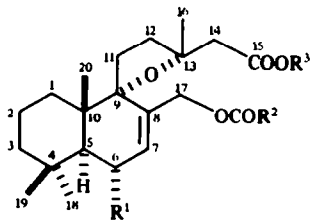
Numerous arid-adapted taxa of the New World genus *Grindelia* are characterized by the abundant production of resinous exudates which cover the surfaces of the leaves, involucres of the flower heads and stems. The resins of *G. camporum* contain bicyclic grindelane diterpenoids which have physical properties that closely resemble those of the acids found in pine wood resin. These grindelanes are being investigated for the possibility of developing the resins of *Grindelia* as substitutes for wood resin used in the naval stores industry [1].

In continuation of our phytochemical investigations of the genus *Grindelia*, we now report the isolation and identification of 17 diterpene acids, as their methyl ester derivatives from the xerophytic *G. acutifolia* Steyerm. from New Mexico. Five of the labdanoids are new natural products and were identified as 6α-hydroxy-17-acetoxygrindelic acid (**13a**), 6α-hydroxy-17-isovaleroxygrindelic acid (**14a**), 6α-hydroxy-17-(α-methylbutyroxy)grindelic acid (**15a**), 6-oxo-17-acetoxygrindelic acid (**16a**) and 6-oxo-17-isobutyroxygrindelic acid (**17a**).

RESULTS AND DISCUSSION

TLC and quantitative GC analyses of the methyl ester mixture of the acid fraction of *G. acutifolia* gave chromatograms which indicated close similarity to the methyl ester mixture from *G. camporum* [2]. However, in *G. camporum*, methyl grindelate was the major constituent, whereas methyl 17-acetoxygrindelate was predominant in *G. acutifolia*. Moreover, *G. acutifolia* lacked methyl isogrindelate and methyl labd-6,8(17)-diene, and contained five new diterpenoids.

Silica gel chromatography of the methyl ester mixture of *G. acutifolia*, eluting with *n*-hexane–ethyl acetate gradients, gave 17 grindelane diterpenoids (**1b**–**17b**) which included previously reported methyl grindelate (**1b**) and its 6α-hydroxy (**2b**), 6-oxo (**3b**) and 7α,8α-epoxy (**4b**) derivatives, methyl strictanonoate (**5b**), five 17-substituted homologues [hydroxy (**6b**), methoxy (**7b**), acetoxy (**8b**), propionoxy (**9b**) and isobutyroxy (**10b**)], methyl 18-isobutyroxy-(**11b**) and 18-isovaleroxy- (**12b**) grindelates [2–5], and five new grindelane diterpenoids: methyl 6α-hydroxy-17-acetoxygrindelate (**13b**), methyl 6α-hydroxy-17-isovaleroxygrindelate (**14b**), methyl 6α-hydroxy-17-(α-methylbutyroxy)grindelate (**15b**), methyl 6-oxo-17-acetoxygrindelate (**16b**) and methyl 6-oxo-17-isobutyroxygrindelate (**17b**). Compounds **1b**, **2b**, **4b**, **5b**, **7b**, and **9b** were identified by direct retention time comparisons with authentic samples according to our standard procedures [2], and the other compounds were characterized spectroscopically. With the availability of additional NMR spectra of grindelanes, it now appears that several previous assignments were incorrect. Three pairs of shift values were reversed for **3b** in ref. [3]: C-1



	R¹	R²	R³
13a	OH	Me	H
13b	OH	Me	Me
14a	OH	*iso*-Bu	H
14b	OH	*iso*-Bu	Me
15a	OH	*sec*-Bu	H
15b	OH	*sec*-Bu	Me
16a	=O	Me	H
16b	=O	Me	Me
17a	=O	*iso*-Pr	H
17b	=O	*iso*-Pr	Me

with C-12, C-7 with C-8, and C-17 with C-19. In ref. [4], the reported ^{1}H NMR shifts of H-7, the upfield H-17 and H-2′ for **10b** should be δ 5.92, 4.59 and 2.56, respectively, and the ^{1}H shift of H-2′ for **11b** should be δ 2.55. Several compounds were not obtained pure but their structures were evident from the spectral data described below on the mixtures in which they occurred: (1) **10b** (30%)–**11b** (55%)–**12b** (15%); (2) **14b** (70%)–**15b** (30%); (3) **3b** (30%)–**17b** (70%). This was of considerable help in assigning ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) parameters since the amount of the substances in each mixture was always quite different. The previously unreported ^{13}C NMR parameters of **10b–12b** are included in Table 2.

Compound **16b**, mp 130–131°, $[\alpha]_D^{25}$ −73.7° (*c* 1.6; chloroform), was found to have molecular weight 406 ($[M]^+$) by low-resolution mass spectrometry and molecular formula $C_{23}H_{34}O_6$ by high-resolution mass spectro-

Table 1. ^{1}H NMR chemical shifts (δ, $CHCl_3$–TMS) and coupling constants (Hz, in parentheses) for compounds **13b–17b**

Proton	**13b**	**14b**	**15b**	**16b**	**17b**
5	1.79 *d* (8.9)	1.79 (8.9)	1.79 (8.9)	2.82*s*	2.82
6	4.07 *td* (8.9, 3.5)	4.07 (9.4, 3.1)	4.07 (9.4, 3.1)	—	—
7	5.87 *d* (3.5)	5.87 (3.6)	5.87 (3.6)	5.87 *t* (1.6)	5.86 (1.5)
14a	2.59 *d* (14.0)	2.57 (14.1)	2.56 (14.0)	2.61 (14.5)	2.61 (14.3)
14b	2.64 *d* (14.0)	2.64 (14.1)	2.64 (14.0)	2.71 (14.5)	2.72 (14.3)
16	1.35 *s*	1.35	1.35	1.42	1.43
17a	4.58 *d* (13.2)	4.60 (13.1)	4.60 (13.1)	4.79 *dd* (13.2, 1.6)	4.80 *d* (1.5)
17b	4.66 *d* (13.2)	4.67 (13.1)	4.67 (13.1)	4.80 *dd* (13.2, 1.6)	4.80 *d* (1.5)
18	1.14 *s*	1.14	1.14	1.19	1.19
19	1.01 *s*	1.01	1.01	1.12	1.12
20	0.83	0.83	0.83	0.96	0.97
OMe	3.65 *s*	3.65	3.65	3.66	3.66
2′	2.09 *s*	2.22 ~ *d* (7.0)	2.40 *sextet* (6.9)	2.14 *s*	2.64 *sextet* (7.0)
3′	—	—	1.15 *d* (7.6)	—	1.21 *d* (7.0)
4′	—	0.96 *d* (6.5)	0.91 *t* (7.4)	—	—

Table 2. ^{13}C NMR chemical shifts (δ, $CDCl_3$–TMS) for compounds **10b–17b**

Carbon	**10b**	**11b**	**12b**	**13b**	**14b**	**15b**	**16b**	**17b**
1	32.5	32.3		33.2	33.3		32.5	32.6
2	18.7	17.9		18.9	19.0		18.0	18.0
3	41.9	36.1		42.9	42.9		42.9	43.0
4	33.2	36.9	36.7	33.6	33.7		32.5	32.6
5	42.0	37.3	37.5	51.3	51.4		56.6	56.6
6	24.3	24.3		67.5	67.6		200.1	200.0
7	132.7	125.9		134.2	134.1		127.1	127.1
8	134.6	135.0		136.2	136.6		151.8	152.0
9	89.6	90.2		89.1	89.2		88.8	89.0
10	40.7	40.4		43.9	43.9		45.9	46.0
11	27.7	28.1	28.2	27.1	27.1		27.9	27.9
12	38.2	38.3		38.3	38.4		38.5	38.6
13	81.6	81.3		81.6	81.6		82.9	82.9
14	47.5	47.9		47.2	47.3		47.3	47.4
15	171.4	171.6		171.3	171.3		171.1	171.0
16	27.2	27.4		27.4	27.4		27.5	27.5
17	66.3	17.8*		65.1	64.9		62.3	62.3
18	32.7	72.8	72.5	35.2	35.2		33.5	33.5
19	22.0	21.1*		22.6	22.6		21.6	21.6
20	16.7	17.1		18.8	18.9		19.8	19.8
OMe	51.1	51.1		51.5	51.6		51.5	51.5
1′	176.5	176.7	171.4	170.5	171.3	172.6	170.2	176.2
2′	34.1	34.2	43.7	20.9	43.5	41.2	20.8	34.0
3′	18.8	18.9	25.5	—	25.6	26.7	—	18.9
4′	—	—	22.4	—	22.4	11.5	—	—
5′	—	—	—	—		16.4	—	—

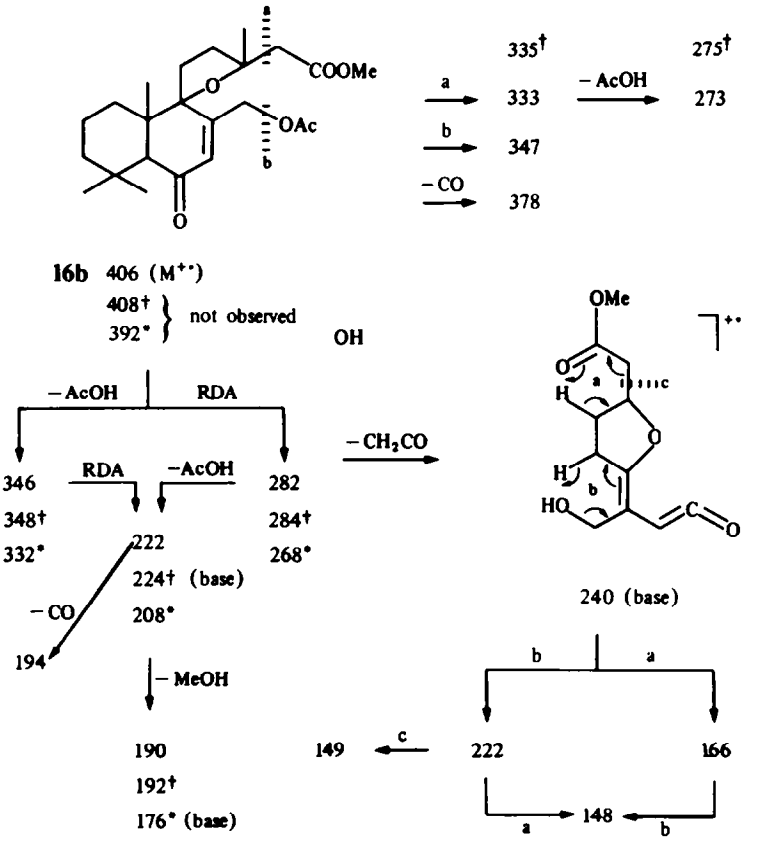
* May be reversed.

metry. The IR ($CHCl_3$) spectrum of **16b** showed three carbonyl bands, two ester (1752 and 1745 cm^{-1}) and one conjugated (1665 cm^{-1}), in addition to C=C (870 cm^{-1}) and $-C(Me)_2-$ (1388 and 1368 cm^{-1}) groupings and a lack of hydroxyl group absorption.

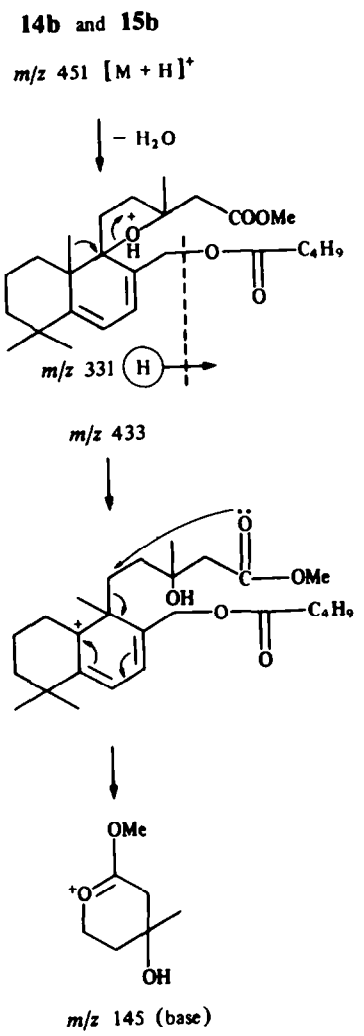
The low-resolution mass spectrum of **16b** exhibited a discernible $[M]^+$ peak at m/z 406 (seen more clearly in its high-resolution mass spectrum). The fragmentation pattern (Scheme 1; molecular formulae of all fragments shown were verified by high-resolution mass spectrometry), except for variations in the intensitities of peaks, was very much like that of **8b**. The difference of 14 mu in many of the peaks of **16b** and **8b** and comparison of their molecular formulae suggested that one of the $-CH_2-$ groups was replaced by a C=O group in **16b**. From the loss of 124 mu from $[M]^+$ (m/z 282) and $[M-HOAc]^+$ (m/z 222) via retro-Diels–Alder breakdown, which clearly suggested that ring A is unsubstituted, the location of the C=C bond was readily perceived. The very low frequency of one of the carbonyl groups (1665 cm^{-1}) clearly indicated conjugation with the C=C bond, a conclusion which was supported by the transition m/z 222 ($C_{12}H_{14}O_4$) $\xrightarrow{-CO}$ m/z 194 ($C_{11}H_{14}O_3$) and confirmed by finding the C-8 absorption unusually far downfield in the ^{13}CNMR spectrum (Table 2). That the oxidation level at C-6 was the only difference between **16b** and **8b** was clear from examination of all of the spectra.

The IR ($CHCl_3$) spectrum of **13b**, $[\alpha]_D^{25} - 41.0°$ (*c* 5.8; chloroform), like **16b** and **8b** showed bands for ester carbonyl (1730 cm^{-1}, broad) and C=C (3030 and 855 cm^{-1}) groupings, but unlike **16b** lacked a conjugated carbonyl band. Instead, hydroxyl absorption (3430 cm^{-1}) was observed. This suggested the possibility of **13b** being a dihydro product of **16b** in which the ketone group was reduced to >CH–OH, an inference which was supported by the low-resolution mass spectrum and confirmed by ^1HNMR (Table 1) and ^{13}CNMR (Table 2) studies. Compound **13b** did not display a $[M]^+$ peak but did show diagnostic peaks at m/z 348, 335, 284 and 224 (base peak) from which the $[M]^+$ peak was inferred to be at m/z 408 as shown in Scheme 1. Comparison of the ^1HNMR and ^{13}CNMR absorptions with those of **2b** and **8b** clearly show that this new compound is **13b**, with a 6α-hydroxyl group.

The structures of **14b** and **15b** followed from comparisons of their ^1HNMR and ^{13}CNMR and FAB mass spectra with those of **13b**. The IR (CCl_4) spectrum displayed strong hydroxyl absorption. The presence of a saturated 5-carbon ester grouping followed from the FAB mass spectrum (m/z 451 $[M+H]^+$) where a fragment $[MH-H_2O]^+ - C_4H_9COOH$ (m/z 331) was visible.



Scheme 1. Major fragment ions (m/z ratios) in the mass spectrum of **16b**. *In **8b**; † in **13b**.



Scheme 2.

The base peak was at m/z 145; possible formulation is shown in Scheme 2. The ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2) clearly showed a mixture of 70% *iso*-butyrate (**14b**) and 30% secondary butyrate (**15b**) groupings at C-17, with the rest of the molecule being as in **13b**.

Compound **17b**, like **16b**, had a strong IR (CCl_4) band for conjugated ketone at 1680 cm^{-1} and lacked hydroxyl absorption. Its structure was deduced largely from NMR comparisons with **10b** and **16b**. The FAB mass spectrum of **17b**, like **14b** and **15b**, gave a base peak at m/z 145 and a strong peak at m/z 347 ($[M+H]^+ - C_3H_7COOH$) indicating the presence of a saturated 4-carbon ester grouping.

EXPERIMENTAL

General. For experimental procedures, see refs. [6, 7].

Plant material. The plant material used in this study was collected in New Mexico, Colfax County, along NM 72, 7.4 miles east of NM 25, on the west side of Johnson Mesa (BNT and DW 847). A herbarium specimen has been deposited at the University of Arizona. All plant material was air-dried, ground to 3 mm particle size and stored at 5° prior to extraction.

Extraction. The milled aerial parts of *G. acutifolia* (492 g) were extracted exhaustively with CH_2Cl_2 in a Soxhlet extractor. The extract was stripped of the solvent and subsequently stored at 5° before work-up. The CH_2Cl_2 extract (42.9 g) was extracted by stirring with MeOH (600 ml) at room temp. (4 hr), left in a refrigerator overnight and filtered. The filtrate was vacuum-dried, dissolved in Et_2O, precipitated with petrol, and left in a refrigerator overnight. The mother liquor was combined with the mother liquor obtained after treating the ppt. with Et_2O. The combined, dried extracts corresponding to the mother liquors (30.7 g) were separated into neutral and acidic (23.5 g) fractions [2]. The latter fraction was methylated according to ref. [2], producing 23.7 g of a methyl ester mixture.

Chromatography. The methylated acid mixture (23.6 g) was chromatographed on silica gel 60 (800 g) eluting the column initially with *n*-hexane–EtOAc (24:1) followed by gradually increasing the concn of EtOAc to 100%. Fifty-five fractions of various volumes were collected.

Isolation of compounds **13b–17b.** Compound **16b** was isolated from fraction 51 by repetitive prep. TLC, *n*-hexane–EtOAc (3:1, multiple developments, for first and second TLC) and *n*-hexane–toluene–Me_2CO (20:10:3, multiple developments, for final TLC). Compound **13b** was isolated from fraction 52 by repetitive prep. TLC, *n*-hexane–EtOAc–HOAc (30:5:1, multiple developments, for first TLC), *n*-hexane–EtOAc (2:3, for second and final TLC). Compound **17b** was isolated from fractions 42–45 by repetitive prep. TLC using EtOAc–HOAc (10:1) with various concns of *n*-hexane (60–40). Compounds **14b** and **15b** were isolated as a mixture from fraction 51 by repetitive prep. TLC developing with the same solvent systems used for **16b**.

The physical and spectral properties of **13b–17b** have already been discussed in the text.

Acknowledgements—We thank Mr. Pramuk Shivanonda for technical assistance, Mr. Peter Baker for mass spectral data and Dr. David Wright for assistance during the plant collecting trips. This investigation was supported by Grant PCM-8304771 from the NSF, whom we gratefully acknowledge.

REFERENCES

1. Timmermann, B. N. and Hoffmann, J. J. (1985) in *Plants for Arid Lands* (Wickens, G. E., Goodin, J. R. and Field, D. V., eds.) pp. 357–368. George Allen & Unwin, London.
2. Timmermann, B. N., Luzbetak, D. J., Hoffmann, J. J., Jolad, S. D., Schram, K. H., Bates, R. B. and Klenck, R. E. (1983) *Phytochemistry* **22**, 523.
3. Hoffmann, J. J., McLaughlin, S. P., Jolad, S. D., Schram, K. H., Tempesta, M. S. and Bates, R. B. (1982) *J. Org. Chem.* **47**, 1725.
4. Bohlmann, F., Ahmed, M., Borthakur, N., Wallmeyer, M., Jakupovic, J., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 167.
5. Timmermann, B. N., Hoffmann, J. J., Jolad, S. D., Schram, K. H., Klenck, R. E. and Bates, R. B. (1982) *J. Org. Chem.* **47**, 4114.
6. Timmermann, B. N., Hoffmann, J. J., Jolad, S. D. and Schram, K. H. (1985) *Phytochemistry* **24**, 1031.
7. Jolad, S. D., Hoffmann, J. J., Schram, K. H., Cole, J. R., Tempesta, M. S. and Bates, R. B. (1981) *J. Org. Chem.* **46**, 4267.